



Evenness of Dietary Protein Intake Is Positively Associated with Lean Mass and Strength in Healthy Women

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ABSTRACT

BACKGROUND: Evenness of protein intake is associated with increased lean mass, but its relationship with muscle strength and performance is uncertain.

OBJECTIVES: We determined the association of evenness of protein intake with lean mass, muscle strength and endurance, and functional ability.

DESIGN: This was a cross-sectional study.

SETTING: Data were collected at a research university in the upper midwestern United States.

PARTICIPANTS: One hundred ninety-two healthy women, aged 18 to 79 years, mean \pm SEM 41.9 \pm 1.3, completed the study.

MEASUREMENTS: Dietary intake was assessed using 3-day food diaries verified with food frequency questionnaires. To assess evenness of protein intake, the day was divided into 3 periods: waking to 11:30, 11:31 to 16:30, and after 16:30. Lean mass was measured with dual energy X-ray absorptiometry. Lower-body muscle strength and endurance were determined using isokinetic dynamometry. Upper-body muscle strength was maximal handgrip strength. Functional ability was assessed using 6-m gait speed and 30-second chair stand tests. Accelerometry measured physical activity.

RESULTS: Intakes of 25 g or more of protein at 1 or more of the 3 periods was positively associated with lean mass ($\beta \pm$ S.E.; 1.067 \pm 0.273 kg, $P < .001$) and upper-body (3.274 \pm 0.737 kg, $P < .001$) and lower-body strength (22.858 \pm 7.918 Nm, $P = .004$) when controlling for age, body mass index, physical activity, and energy and protein intakes. Consuming at least 0.24 g/kg/period for those under 60 years and 0.4 g/kg/period for those 60 years and older was related to lean mass (0.754 \pm 0.244 kg, $P = .002$), upper-body strength (2.451 \pm 0.658 kg, $P < .001$), and lower-body endurance (184.852 \pm 77.185 J, $P = .018$), controlling for the same variables.

CONCLUSIONS: Evenness of protein intake is related to lean mass, muscle strength, and muscular endurance in women. Spreading protein intake throughout the day maximizes the anabolic response to dietary protein, benefiting muscle mass and performance.

KEYWORDS: Protein distribution, muscle mass, muscular endurance, muscle strength

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Introduction

Skeletal muscle mass comprises 40% to 50% of body mass¹ and contains approximately 45% of the human body's total protein content.² Muscle tissue acts as an "amino acid reservoir,"^{3,4} catabolizing itself to provide amino acids or energy to other tissues after traumatic injuries or infections⁵ or during periods of negative energy balance.⁶ Naturally then, sarcopenia, a condition

characterized by reduced muscle quantity and strength, is related to both an increased risk of disability and all-cause mortality.⁷ Increasing or maintaining muscle quantity and strength is important throughout the lifespan, as is indicated by both experts^{8,9} and the United States Department of Health and Human Services,¹⁰ yet muscle mass and strength decline as individuals age.¹¹⁻¹³



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Having adequate dietary intake represents a relatively well-tolerated and low-cost method to mitigate losses of muscle quantity and strength associated with aging, bedrest, or trauma.¹⁴⁻¹⁷ In addition to the detrimental effects of aging on muscle strength and quantity, an individual's ability to taste decreases with aging¹⁸ as does one's oral health¹⁹ and ability to masticate.²⁰ As the result of these changes, among other factors, dietary intake decreases by about 25% from age 40 to 70 and predisposes middle-aged and older adults to malnutrition which can hasten the development of sarcopenia.²¹ Several nutrients are particularly important for preserving muscle quantity and strength including protein, fatty acids, vitamin D, antioxidants, and minerals such as iron, magnesium, calcium, selenium, and zinc.^{16,22}

Beyond being the "building-blocks" of proteins, dietary amino acids contribute to muscle protein synthesis by activating the mammalian target of rapamycin complex 1.^{23,24} This makes dietary protein of particular interest because of the nutrient's ability to directly affect muscle protein synthesis and breakdown.^{17,23,25,26} In fact, about 25 to 30 g of protein is the amount required for muscle protein synthesis,²⁶ and it is thought that by achieving intakes of this amount more frequently, such as at each meal, one would maximize muscle protein synthesis, benefiting muscle mass and strength.²⁷ In support of this notion, the primary estimation studies of nitrogen balance that informed the National Institutes of Health 0.8 g/kg body weight per day recommendation for dietary protein intake only included studies where all participants ate at least 3 meals,^{28,29} guaranteeing some level of evenness in dietary protein spread.

A systematic review of 15 studies investigating the evenness of dietary protein intake concluded there was enough evidence to determine that evenness of protein intake distribution was related to increased muscle mass, but there was not enough evidence to determine its effects on muscle strength or protein turnover.²⁷ Considering this conclusion, we sought to determine the association of evenness of dietary protein intake with lean mass, muscle strength and endurance, and functional ability. Other investigators of dietary protein intake distribution have not controlled for energy intake,³⁰⁻³³ which is critical to include in statistical models investigating nutritional variables.^{27,34} Moreover, some of these groups,^{30,32,33} when investigating dietary protein intake distribution, did not control for total, relative, or percent of energy from protein intake, which can also affect muscle mass and performance.^{35,36} Additionally, the authors of the systematic review advocate for cut-points of 0.24 g/kg body weight per meal for younger adults and 0.4 g/kg per meal for older adults,²⁷ as these cut-points were informed by a breakpoint analysis of muscle protein synthesis data between healthy younger and older men.³⁷ However, as there is lack of consensus regarding how to measure or define dietary protein intake distribution,²⁷ we sought to compare the previous recommendation of 25 to 30 g of protein per meal,

the minimum amount thought to elicit a maximal anabolic response,²⁶ to these newer relative cut-points in a population of healthy women.

Methods

This project was conducted in the North Dakota State University Healthy Aging Research Lab from October 2017 to December 2019. A total of 195 women from the local community were recruited using e-mail, flyers, and word-of-mouth to visit the research lab for 2 sessions. During the first session, anthropometric and performance variables were measured, and accelerometers, 3-day food diaries, and food frequency questionnaires (FFQ) were provided.³⁸ Within 7 to 14 days later, participants returned to the lab to return their accelerometers, food diaries, and FFQs and have a full-body dual energy x-ray absorptiometry (DXA) scan performed. Participants were between 18 and 80 years of age, not currently using any nicotine products, free of any untreated or nonresponsive diseases or conditions, ambulatory without any assistance, and had to include both animal-based and plant-based foods in their diets. Those who reported working during the night were excluded. Participants were eligibility screened using the 2017 Physical Activity Readiness Questionnaire,³⁹ a more detailed health history questionnaire, and an orthostatic hypotension test. The study was approved by the North Dakota State University Institutional Review Board and complied with the Helsinki Declaration of 2013.

Participant Health Screening and Anthropometric Measures

To screen participants for orthostatic hypotension, related to regulatory and safety concerns set forth by the Institutional Review Board, resting blood pressure and standing blood pressure were measured manually with a stethoscope and Diagnostix 703 sphygmomanometer (American Diagnostic Corporation, Hauppauge, NY). Those whose blood pressure dropped by more than 10 mmHg, either systolic or diastolic, from resting to standing during the orthostatic hypotension test were excluded (n=0). Following the orthostatic hypotension test, anthropometric variables were measured. Age (years) was self-reported. Height, to the nearest mm, was measured using a stadiometer (Seca 213, Chino, CA) and body mass, to the nearest 0.1 kg, was recorded using a digital balance scale (Denver Instrument DA-150, Arvada, CO). Waist and hip circumferences were completed using a Gulick (Fitness Mart Division of Country Technology Inc., Gays Mills, WI) spring-loaded measuring tape to the nearest mm.

Performance Measures

Prior to performance testing, participants completed a light, self-paced, 5-minute warm-up on a cycle ergometer. To optimize performance, research staff encouraged participants to

employ “all-out effort” during tests of muscle strength and endurance. Handgrip strength (kg) was assessed first using an analog Jamar Handheld Dynamometer (Bolingbrook, IL). Each participant was instructed to grasp the dynamometer in her dominant hand and to keep her elbow at her side with a 90° bend between the upper arm and forearm while standing. Participants were told to squeeze the dynamometer as hard as possible for 2 to 3 seconds. Each participant performed 3 maximal attempts; the highest grip strength was used. Gait speed was then measured using a Brower TCi system (Draper, UT). Participants were instructed to walk at their normal pace over a 10 m distance. Timing gates were placed 6 m apart. Gait speed was recorded 3 times, and mean time was used in analyses. Participants then performed a 30 seconds chair stand test on a 43 cm chair. All trials were performed with participants’ arms crossed and feet at a comfortable distance apart (ie, about hip to shoulder width). Participants were instructed to fully sit down and stand up for each repetition, and practice repetitions were performed to ensure adequate performance during the test. The total number of repetitions completed in 30 seconds was recorded. Participants were seated, and the 30 seconds period began when participants started to rise.

After these 3 assessments, muscle strength and endurance of the lower-body were tested using isokinetic dynamometry on a Biodex Pro IV System (Biodex Medical Systems, Shirley, NY). Lower-body muscular strength was assessed using peak torque performed during a 3-repetition test at 60°/second for knee extension-flexion and a 3-repetition test at 30°/second for plantar-dorsiflexion. Similar to others’ work,⁴⁰ lower-body muscular endurance was evaluated using the total amount of work performed during a 21-repetition test at 180°/second for knee extension-flexion and 60°/second for plantar-dorsiflexion. Muscular strength and then endurance were first assessed in the upper leg (ie, knee extension-flexion) and then in the lower leg (ie, plantar-dorsiflexion). A warm-up set was completed before each lower-body strength test (ie, knee extension-flexion and plantar-dorsiflexion); participants were instructed to perform 3 repetitions at <75% of their perceived maximal effort. Thirty seconds of rest was given between all extension-flexion tests. One minute of rest was provided between plantar-dorsiflexion tests. To better capture muscular performance of the entire right leg, peak torques from the isokinetic strength test and total work from the isokinetic muscular endurance test were added together to create summed peak torque and summed total work (ie, knee extension + knee flexion + plantarflexion + dorsiflexion).

Physical Activity Assessment

Following performance testing, accelerometers, 3-day food diaries, and FFQs were given to participants. Physical activity was recorded using Actigraph (Pensacola, FL) GT9X accelerometers worn on the non-dominant wrist for 7 consecutive days. Participants were instructed to wear the accelerometer

during all waking hours except activities involving water (eg, bathing or swimming). The raw acceleration data were collected at 80 Hz and processed in R software using the GGIR package (version 1.10-10).⁴¹ A sleep log was provided to help delineate non-wear time from time spent sleeping. Non-wear time was defined as intervals of at least 90 minutes of zero counts with allowance of 2-minute interval of non-zero counts within a 30 minutes window,⁴² thus only valid time during waking hours of each day was included for statistical analyses. The minimum number of wear days was 4, including 1 weekend or 1 non-routine day, over the weeklong collection period, with a minimum wear time of 10 hours/day. Due to its beneficial,⁴³⁻⁴⁵ but in this case, also confounding effect on muscle and performance, moderate to vigorous physical activity (MVPA) was included in all analyses as a covariate.

Nutrition Analysis

Participants were given both 3-day food diaries and a 153-item FFQ³⁸ and received training on how to record dietary intakes by a member of the research team. Participants were also required to watch a prerecorded training video provided by the study’s registered dietitians. Dietary intakes from 3-day food diaries, including nutritional supplements, were entered into Food Processor Nutrition Analysis Software (ESHA Research, Salem, OR) which uses Food Data Central (ie, the USDA Nutrient Data Base),⁴⁶ by trained research assistants. Data entry was then line-by-line verified by a registered dietitian. As the 3-day food diary asked participants to record their intakes in real-time and the FFQ asked participants about their intake over the last 90 days, the methods do not assess the same nutritional variables; the former represents immediate intake, whereas the latter represents some level of historical intake. Nonetheless, as this project lacked criterion validity for dietary intake (ie, an objective measure of dietary intake was not performed),⁴⁷ the data from the FFQ was used as to verify estimates from 3-day food diaries.^{48,49}

Follow-up visit

After 7 to 14 days, participants returned to the lab to turn in accelerometers, food diaries, and FFQs, have their lean mass and percent body fat measured, and give a blood sample. Lean mass and percent body fat measured were measured via DXA on a Lunar Prodigy model #8915 (GE Healthcare, Waukesha, WI), with enCORE software.

Statistical Analyses

A total of 192 women completed both a 3-day food diary and the FFQ and wore an accelerometer for at least 10 hours a day for 4 or more days. Three participants were excluded from all analyses because they failed to wear the accelerometer as directed. Thus, all analyses have at most 192 participants.

Descriptive statistics including self-reported age, BMI, and MVPA were reported as means and standard errors of the means. Total and relative intakes, including the percent of energy from each of the macronutrients, were verified using paired *t*-tests between data from the 3-day food diary and the FFQ. Mean total (eg, g/day) and relative (eg, g/kg/day) dietary intake of energy, carbohydrate, fat, and protein and the percentages of energy from carbohydrate, fat, and protein are listed for both the 3-day food diary and the FFQ in addition to their mean paired differences.

To examine the effects of the evenness of protein intake distribution, data collected from 3-day food diaries were first blocked into 3 periods: waking to 11:30 (breakfast), afternoon 11:31 to 16:30 (lunch), and evening after 16:30 (dinner). Protein intake was averaged for each period across all 3 days that the food diary was recorded, and mean protein intake for each period was listed with 95% confidence interval for exploratory comparisons among periods. Even protein intake distribution was then defined using 2 methods: a relative intake methodology (ie, 0.24 or 0.4 g/kg body weight or more per period) and a total intake methodology (ie, 25 g or more per period). Mean relative protein intakes of at least 0.24 g/kg of body weight per period for younger adults (<60 years) and 0.4 g/kg body weight per period for older adults (≥ 60 years), respectively, were the cut-points for the relative intake method,^{27,37} whereas greater than or equal to 25 g/period was the cut-point for total intake method;²⁶ consuming an average of protein equal to or greater than these cut-points during one of these periods were recorded as “1s”, and these were summed to create 2 ordinal variables each with 4 levels, achieving greater than 0.24/0.4 g/kg body weight per period or 25 g/period at 0, 1, 2, or 3 periods. These ordinal variables were entered into 2 separate multiple linear regression models each controlling for age, BMI, MVPA, relative energy intake, and percent of energy from protein. The outputs of interest for these models were the betas associated with dietary protein intake distribution and the overall significance of these models.

Results

Table 1 displays descriptive statistics for the 192 women included in this work.

All analyses except for that of the 30 seconds chair stand test had 192 participants; the chair stand test had 191 participants. Age ranged from 18 to 79 years. A total of 147 participants (76.6%) were less than 60 years of age, and 45 (24.4%) were 60 years or older. The minimum BMI was 15.3 and the maximum was 41.9 kg/m². According to BMI, 2 participants (1.0%) were underweight with BMIs less than 18.5 kg/m², 96 (50%) had BMIs between 18.5 and less than 25, 63 (32.8%) were overweight with BMIs between 25 and less than 30 kg/m², 20 (10.4%) had BMIs between 30 and less than 35 kg/m², 9 (4.7%) had BMIs between 35 and less than 40 kg/m², and 2 had (1.0%)

Table 1. Descriptive statistics of the 192 women included in this work.

| VARIABLES | MEAN \pm SEM |
|--------------------------|-----------------|
| Age (y) | 41.9 \pm 1.3 |
| Height (cm) | 164.8 \pm 0.5 |
| Body mass (kg) | 70.0 \pm 1.0 |
| BMI (kg/m ²) | 25.7 \pm 0.3 |
| MVPA (min/d) | 89.3 \pm 2.2 |

Abbreviations: BMI, body mass index; MVPA, moderate to vigorous physical activity; SEM, standard error of the mean.

had BMIs of 40 or greater kg/m²; thus, 31 participants (16.1%) were considered obese according to BMI. A total of 171 (89.1%) of participants wore accelerometers for at least 7 days with greater than or equal to 10 hours of wear time on each day (a valid wear day was considered to have to 10 hours of wear time); 1 participant (0.5%) only had 4 days, 3 (1.6%) had 5 days, and 17 (8.9%) had 6 days with at least 10 hours of wear time. Time spent in MVPA ranged from a minimum of 18.8 and a maximum of 185.9 minutes/day.

The results of the paired *t*-test analyses of dietary intake data comparing the 3-day food diary and the FFQ are shown in Table 2.

One of the goals of this work was to control for both energy and protein intakes when investigating dietary protein distribution, as recommended by others,^{27,34} yet relative energy and protein intakes are related,³⁰ potentially biasing estimates when entered into the same statistical model.⁵⁰ In models evaluating dietary protein intake distribution, energy intake was expressed as relative energy intake (ie, kcal per kg body weight per day) and protein intake as a percentage of energy intake. As percent of energy from carbohydrate and fat were different according to paired *t*-test analyses of data from the 3-day food diaries and the FFQs, these variables were not used as covariates in regression models.

The distribution of dietary protein intake is described in Table 3. Intakes were greatest in the evening or dinner period and lowest during the morning or breakfast period. Of the 147 participants less than 60 years of age, 67 (45.6%), 116 (78.9%), and 143 (97.3%) consumed an average of at least 0.24 g of protein per kg body weight during the breakfast, lunch, and dinner periods, respectively. Of the 45 participants 60 years and older, 13 (28.9%), 22 (48.9%), and 33 (73.3%) consumed an average of at least 0.4 g of protein per kg body weight during the breakfast, lunch, and dinner periods, respectively. For the relative protein intake per period summed ordinal variable, 9 (4.7%) participants had a score of 0 (<60 years = 2; ≥ 60 years = 7), 34 (17.7%) had a score of 1 (<60 years = 19; ≥ 60 years = 15), 87 (45.3%) had a score of 2 (<60 years = 71; ≥ 60 years = 16), and 62 (32.3%) had a score of 3 (<60 years = 55; ≥ 60 years = 7).

Table 2. Paired comparison of dietary intake data from 3-day food diaries and the food frequency questionnaire.

| VARIABLES | THREE-DAY DIARY | FFQ | PAIRED DIFFERENCE | |
|---------------------------------|-----------------|----------------|-------------------|-------|
| | MEAN ± SEM | MEAN ± SEM | MEAN ± SEM | P |
| Total energy (kcal/d) | 2022 ± 40 | 2004 ± 63 | 18 ± 58 | .758 |
| Total protein (g/d) | 85.3 ± 1.8 | 85.2 ± 2.9 | 0.0 ± 2.6 | .989 |
| Total fat (g/d) | 84.5 ± 2.0 | 77.4 ± 2.6 | 7.1 ± 2.5 | .006 |
| Total carbohydrate (g/d) | 230.9 ± 5.7 | 243.5 ± 8.5 | -12.6 ± 7.6 | .099 |
| Relative energy (kcal/kg/d) | 29.736 ± 0.686 | 29.378 ± 0.977 | 0.359 ± 0.858 | .676 |
| Relative protein (g/kg/d) | 1.262 ± 0.033 | 1.245 ± 0.044 | 0.016 ± 0.038 | .669 |
| Relative fat (g/kg/d) | 1.238 ± 0.033 | 1.128 ± 0.039 | 0.110 ± 0.037 | .003 |
| Relative carbohydrate (g/kg/d) | 3.401 ± 0.095 | 3.587 ± 0.133 | -0.187 ± 0.113 | .100 |
| Protein percent energy (%) | 17.3 ± 0.3 | 17.1 ± 0.2 | 0.1 ± 0.3 | .622 |
| Fat percent energy (%) | 37.2 ± 0.5 | 34.8 ± 0.5 | 2.4 ± 0.4 | <.001 |
| Carbohydrate percent energy (%) | 45.0 ± 0.6 | 48.4 ± 0.6 | -3.3 ± 0.6 | <.001 |

Abbreviations: FFQ, food frequency questionnaire³⁸; SEM, standard error of the mean.

Table 3. Distribution of dietary protein intake from 3-day food diaries with unadjusted 95% confidence interval for comparison.

| VARIABLES | PERIOD | | | |
|------------------------------|-------------------------------------|---------------------------------|----------------------------------|---------------------------------|
| | BREAKFAST MEAN ± SEM [95% CI] | LUNCH MEAN ± SEM [95% CI] | DINNER MEAN ± SEM [95% CI] | TOTAL MEAN ± SEM [95% CI] |
| Total protein (g) | 17.4 ± 0.8 [15.9, 18.9] | 28.1 ± 0.9 [26.3, 29.8] | 39.8 ± 1.1 [37.7, 42.0] | 85.3 ± 1.8 [81.6, 88.9] |
| Relative protein (g/kg) | 0.255 ± 0.012 [0.232, 0.278] | 0.418 ± 0.015 [0.388, 0.448] | 0.588 ± 0.018 [0.553, 0.623] | 1.262 ± 0.033 [1.197, 1.326] |
| Percent of energy (%) | 3.5 ± 0.2 [3.2, 3.8] | 5.7 ± 0.2 [5.4, 6.0] | 8.0 ± 0.2 [7.7, 8.4] | 17.3 ± 0.3 [16.6, 17.9] |
| Percent of total protein (%) | 20.0 ± 0.7 [18.6, 21.4] | 33.2 ± 0.8 [31.6, 34.7] | 46.8 ± 0.8 [45.2, 48.4] | 100* |

Abbreviations: 95% CI, 95% confidence interval; SEM, standard error of the mean.

*Standard error and 95% confidence interval could not be calculated as all values were 100.

Although the total cut-off (ie, 25 g/period) did not vary for those younger than 60 years and those 60 years and older, data for the total protein intake per period method are presented for these 2 populations separately for comparison with the relative cut-point method. At the morning or breakfast period, 44 (22.9%) participants consumed 25 g of protein or more (<60 years = 32; ≥60 years = 12), at the midday or lunch period 98 (51.0%) participants, consumed 25 g of protein or more (<60 years = 78; ≥60 years = 20), and at the evening or dinner

period 159 (82.8%) participants consumed 25 g of protein or more (<60 years = 120; ≥60 years = 39). For the total protein intake per period summed ordinal variable which counts how many periods participants consumed a mean protein intake of equal to or greater than 25 g, 17 (8.9%) participants had a score of 0 (<60 years = 14; ≥60 years = 3), 73 (38.0%) had a score of 1 (<60 years = 56; ≥60 years = 17), 78 (40.6%) had a score of 2 (<60 years = 57; ≥60 years = 21), and 24 (12.5%) had a score of 3 (<60 years = 20; ≥60 years = 4).

Table 4. Model summaries of separate multiple linear regression models and coefficients evaluating 2 different methods of defining protein intake distribution when controlling for age, BMI, MVPA, relative energy intake, and percent of energy from protein.

| OUTCOME | PROTEIN INTAKE VARIABLE* | MODEL | | | COEFFICIENT | |
|--|--------------------------|-------|--------------------------------|-------|------------------|-------|
| | | R | R ² _{ADJ.} | P | B ± SE | P |
| Lean mass (kg) | ≥25g/period | .710 | .489 | <.001 | 1.067 ± 0.273 | <.001 |
| | 0.24/0.4 g/kg/period† | .700 | .474 | <.001 | 0.754 ± 0.244 | .002 |
| Percent body fat (%) | ≥25g/period | .835 | .687 | <.001 | -0.715 ± 0.563 | .205 |
| | 0.24/0.4 g/kg/period | .833 | .684 | <.001 | -0.033 ± 0.497 | .948 |
| Maximal handgrip strength (kg) | ≥25g/period | .517 | .243 | <.001 | 3.274 ± 0.737 | <.001 |
| | 0.24/0.4 g/kg/period | .495 | .221 | <.001 | 2.451 ± 0.658 | <.001 |
| Thirty second chair stand test (repetitions) | ≥25g/period | .306 | .064 | .006 | 0.348 ± 0.588 | .555 |
| | 0.24/0.4 g/kg/period | .303 | .062 | .006 | 0.07 ± 0.519 | .893 |
| Mean 6 m gait speed (s) | ≥25g/period | .359 | .100 | <.001 | 0.007 ± 0.073 | .927 |
| | 0.24/0.4 g/kg/period | .380 | .117 | <.001 | -0.119 ± 0.064 | .063 |
| Summed lower-body peak torque (Nm) | ≥25g/period | .583 | .319 | <.001 | 22.858 ± 7.918 | .004 |
| | 0.24/0.4 g/kg/period | .561 | .293 | <.001 | 8.019 ± 7.099 | .260 |
| Summed lower-body muscular endurance (J) | ≥25g/period | .544 | .273 | <.001 | 170.522 ± 88.159 | .055 |
| | 0.24/0.4 g/kg/period | .551 | .303 | <.001 | 184.852 ± 77.185 | .018 |

Abbreviations: BMI, body mass index; MVPA, moderate-to-vigorous physical activity; SE, standard error.

*Mean protein intakes during 3 periods from 3-day food diaries, waking to 11:30 (breakfast), afternoon (lunch) 11:31 to 16:30, and evening after 16:30 (dinner), equal to or greater than the listed cut-offs were coded as "1s" and were then summed to create ordinal levels with 4 levels, meeting the cut-off at 0, 1, 2, or 3 periods.

†For those 60 and under 0.24 g/kg/period; for those 60 and over 0.4 g/kg/period.

The results of separate multiple linear regression models evaluating the relationship between these 2 summed ordinal variables with lean mass (kg) and body fat (%) determined via DXA, handgrip strength (kg), 30 seconds chair stand test (repetitions), mean gait speed (s), and summed lower-body strength (Nm) and muscular endurance performance (J) are presented in Table 4. All models were significant (all $P < .05$). Both methods used to define evenness of dietary protein intake distribution were related to total lean mass and maximal handgrip strength. Neither method was related to 30s chair stand or gait speed performance, although the relative (ie, 0.24/0.4 g/kg/period) intake per period method approached significance ($P = .063$) for gait speed. Intakes of ≥25 g of protein per period were related to lower-body strength ($P = .004$), whereas intakes of 0.24 or 0.4 g of protein per kg body weight per period were associated with lower-body muscular endurance ($P = .018$).

Discussion

In this study, evenness of dietary protein intake distribution was related to lean mass using both the 25 g/period and the 0.24/0.4 g/kg body weight per period cut-points which is consistent with the results of others⁵¹⁻⁵³ and the conclusion of

Jespersen and Agergaard²⁷ who wrote the review regarding the evenness of dietary protein intake and muscle mass, strength, and protein turnover. Our finding further supports the hypothesis that achieving sufficient protein intake at each meal increases net protein balance, resulting in higher levels of lean mass. However, evenness of dietary protein intake was not related to percent body fat, and this is in contrast to the findings of another cross-sectional study with similar methods (ie, 3-day food diaries and DXA).⁵² In that study, those who ate more than an average of 0.24 g of protein per kg body weight per meal for all 3 meals had lower body fat percentage than those who did not eat an average of 0.24 g/kg body weight per meal at all 3 meals.⁵² That experimental group, though, was significantly younger consisting of college-aged participants only, and those authors did not use BMI as a covariate in their statistical models.⁵² Of course, BMI and percent body fat are related, but BMI is not an accurate estimate of body fat percentage, often misclassifying people as overweight or obese.^{54,55} Despite the association of BMI with lean mass and body fat percentage,^{54,55} we found that lean mass was related whereas percent body fat was not related to the evenness of dietary protein intake, indicating that the evenness of protein intake is

important for preserving or increasing lean mass but not for losing or preventing gains in fat mass.

Our work does show that evenness of dietary protein intake was positively associated with muscle strength. More specifically, mean intakes of at least 25 g/period were significantly associated with both upper (ie, handgrip) and lower-body strength, whereas intakes of 0.24 or 0.4 g/kg body weight per period were only related to handgrip strength. We do not believe that this disparity is the result of relative per meal metrics (ie, 0.24 or 0.4 g/kg/period) being generally less informative than total per meal metrics (ie, 25 g/period). Rather, the cut-points for this relative method, 0.24/0.4 g/kg body weight per period, are based on 1 work in young (ie, 18-37 years) and older men (ie, 65-80 years) and did not include middle-aged men.³⁷ As our work evaluated women across much of the adult lifespan (ie, 18-79 years), the true relative cut-points needed for muscle protein synthesis are likely different for our sample. Although sex does not affect muscle protein synthesis at fasting⁵⁶ or after a meal (ie, postabsorptive state)⁵⁷ in younger populations, the anabolic effects of a meal are blunted in older women compared to men.⁵⁸ Thus, older women would likely need to achieve protein intakes greater than what was indicated in older men, which is 0.4 g/kg of body weight per meal.³⁷ Future studies should examine the relative amount of protein needed at 1 meal to stimulate muscle protein synthesis in women, particularly older women.

Additionally, our results indicate that evenness of dietary protein intake distribution is related to lower-body muscular endurance. However, there was a discrepancy between our findings for lower-body muscular endurance and lower-body strength when examining the 2 methods of defining even protein intake distribution. In contrast to our findings for lower-body strength, the relative method of expressing the evenness of protein intake distribution was positively associated with lower-body muscular endurance performance, whereas the total method was not. Yet, the total method, as opposed to that of the relative intake method in the case of the lower-body strength model, approached significance and was closer to the estimate of the relative method than the 2 methods were for lower-body strength. Thus, we do not view this difference as incongruent with our results. In order to have a relative intake of exactly 0.24 g/kg of body weight per period when eating 25 g of protein, one would need to have a body mass of approximately 104 kg. The mean body mass of participants was 70 kg for this study and is 77.5 kg for women 20 years and older in the United States.⁵⁹ Thus, 25 g/period is greater than the relative 0.24 g/kg body weight per period cut-point for 95.9% of the 147 women under 60 years of age included in this study and for the average woman in the United States. Our results suggest that relative intakes greater than 0.24 g/kg of body weight per meal or period are likely needed for women under age 60 to see benefit in lower-body strength, but intakes of 0.24 may be sufficient to benefit lower-body muscular endurance.

Evenness of dietary protein intake was not related to functional ability in our sample. The relative intake method approached significance for mean 6 m gait speed, suggesting some benefit with increased evenness of intake. These measures of functional ability, though, may not be related to performance in younger or middle-aged healthy adults. In the context of the European Working Group's revised consensus,⁸ for instance, measures of physical performance (ie, functional ability) are intended to differentiate between those with sarcopenia and those with "severe sarcopenia." In support of this, others,⁶⁰ using a cross-sectional sample of 409 adults aged 60 to 96 years, reported no relationship between isokinetic leg strength and gait speed in stronger older adults, whereas leg strength was related to gait speed in weaker older adults, when using a quadratic regression model. We hypothesize that given an older population associations between the evenness of dietary protein intake and functional ability would be observed, as protein intakes of ≥ 0.25 g/kg/meal were associated with decreased odds of self-reported functional disability.⁶¹

This study had some limitations. It was a cross-sectional study incapable of establishing causality. The participants may not be representative of the larger population, as convenience recruiting methods were used, and only healthy women were allowed to participate. Protein intakes were high also for the sample, averaging 1.26 g/kg body weight per day. Although research shows benefit with protein intakes as high as 1.6 g/kg/day with resistance training,⁶² and our sample was physically active averaging almost 90 minutes/day of MVPA, it is likely that many participants achieved optimal total protein intake, biasing models to show the benefit of more evenly distributing protein intake. Lastly, subjective, self-reported tools measured dietary intake.

We, however, used 2 subjective dietary tools, a 3-day food diary and a FFQ,³⁸ to verify our results. This is a key strength of our work relative to many others who have only used subjective assessments of dietary intake, as using 2 subjective tools to measure dietary intake is considered a best practice when lacking criterion validity.⁴⁷⁻⁴⁹ Moreover, total and relative intakes of energy, protein, and carbohydrate were not significantly different, showing good convergent validity; only total and relative fat intakes were significantly different. When expressed as percentages of energy intake, fat and carbohydrate intakes were significantly different and protein intake was not. Crucially, only relative energy and percent of energy from protein, 2 measures that were not different according to paired t-tests, were included as covariates in regression models examining protein intake distribution. In addition to this unique strength, we included an objective measure of physical activity in our statistical models. Lastly, unlike other groups who have investigated the evenness of dietary protein intake,³⁰⁻³³ we controlled for both energy and protein intakes.

In conclusion, we find further support for the relationship between the evenness of dietary protein intake and lean mass.

We also present compelling cross-sectional data that the evenness of dietary protein intake is positively associated with muscle strength and muscular endurance, even when controlling for physical activity and energy and protein intakes. Future research needs to establish a relative per meal threshold for women as the current 0.24 and 0.4 g/kg body per meal recommendations²⁷ reflect data from men.³⁷

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